

## STABILIZING THE AMMONIA-NITROGEN CONTENT OF ESTUARINE AND COASTAL WATERS BY FREEZING<sup>1</sup>

In 1950, the Bureau of Commercial Fisheries Biological Laboratory at Galveston, Texas, began to investigate methods of stabilizing the chemical composition of seawater samples. The object was to find a noncontaminating means of storing perishable marine and estuarine water samples gathered for chemical analysis. Such a procedure would eliminate the need for immediate analysis and allow storage until the facilities of the shore-based laboratory could be employed.

Since undertaking the investigation, it has been shown that many of the constituents of seawater are effectively stabilized if the samples are placed in cold storage (below freezing) as soon as possible following collection. Collier and Marvin (1953) demonstrated that the concentration of inorganic and organic phosphate in frozen seawater samples remains unchanged for long periods of time. May's (1960) investigation of the cold-storage technique showed that the concentration of carbohydrate in frozen seawater remains stable for about 7 weeks, but that significant

changes then begin to occur. The technique also proved useful in the storage of seawater samples collected for nitrite analysis (Proctor 1962).

This paper presents results showing that ammonia-nitrogen ( $\text{NH}_3\text{-N}$ ) concentration in estuarine and coastal waters, normally containing many times the  $\text{NH}_3\text{-N}$  concentration of open seawater, can also be stabilized by freezing.

Fig. 1 depicts variation with time in the  $\text{NH}_3\text{-N}$  content of estuarine water analyzed under different experimental conditions. All  $\text{NH}_3\text{-N}$  estimates were made using Treadwell's Nessler reagent, recommended for seawater analyses by Wirth and Robinson (1933). Direct Nesslerization was possible by following the procedure of Wattenberg (1931). The curves in Fig. 1A represent the  $\text{NH}_3\text{-N}$  content of a 20-liter sample taken from a small lagoon located at the east end of Galveston Island. At the beginning of the experiment, the  $\text{NH}_3\text{-N}$  content was very low. The sample was therefore fortified with  $(\text{NH}_4)_2\text{SO}_4$  to the  $\text{NH}_3\text{-N}$  level shown, so that variation with time of the  $\text{NH}_3\text{-N}$  concentration could be more easily followed. The sample was fil-

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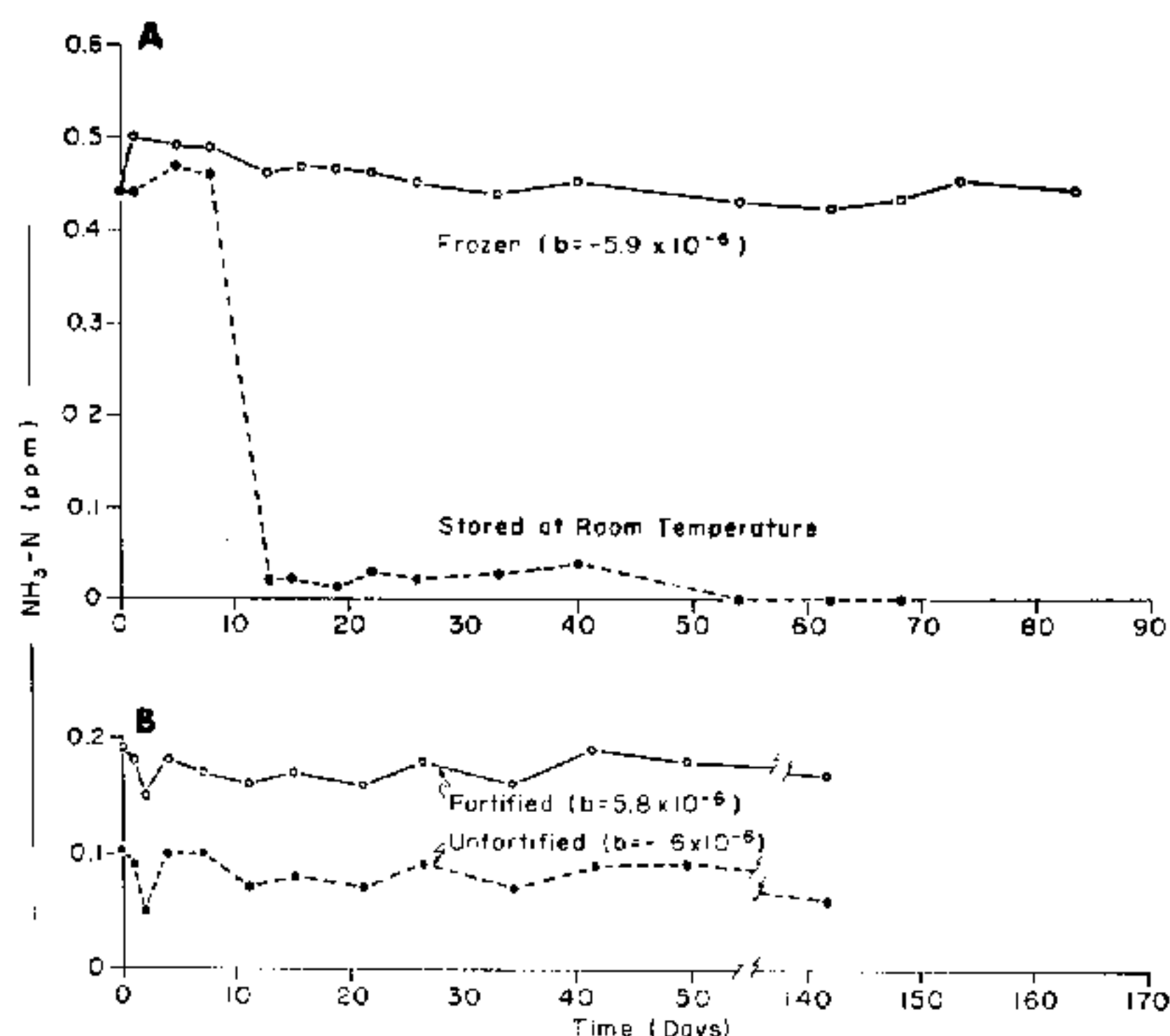


FIG. 1. A. Comparison of  $\text{NH}_3\text{-N}$  stability in frozen and unfrozen estuarine water samples. B. Variation due to systematic errors in measuring the  $\text{NH}_3\text{-N}$  content of frozen coastal water samples.

tered to remove particulate matter and then dispensed into 200-mm glass culture tubes with Poly-Seal caps. Half of the 200 subsamples were stored in a freezer at  $-23^\circ\text{C}$ , and the other half in daylight at room temperature. Each point on the trend curves in Fig. 1A is the average of 5 analyses (5 tubes) made at the indicated intervals.

The curves in Fig. 1B show similar data based on a 20-liter sample of coastal water collected near Galveston Island. These curves demonstrate a source of systematic error and also show that the stabilizing effect of freezing on the  $\text{NH}_3\text{-N}$  content of fortified coastal water and non-fortified water is the same. Thus, the use of  $(\text{NH}_4)_2\text{SO}_4$  to fortify the  $\text{NH}_3\text{-N}$  concentration of the samples investigated was warranted (Fig. 1A). In this experiment, only half the original sample was fortified with  $(\text{NH}_4)_2\text{SO}_4$ . Both halves were filtered, however, and then subdivided as before and stored in a freezer. Each value on Fig. 1B also represents the average of 5 analyses.

A statistical study of the data shown in Fig. 1B suggested that the precision of each estimated value, in terms of the calculated standard deviations, averaged 0.013 ppm. The conventional "F" test revealed further that the curves' fluctuations are statistically significant. The striking similarity of the

curves in Fig. 1B indicates that the observed variation in each is due more to systematic errors or biases within successive sets of subsample measurements than to changes in the  $\text{NH}_3\text{-N}$  concentration of the frozen material. This interpretation is substantiated by the overall rates of change in  $\text{NH}_3\text{-N}$  which are represented by the coefficients of regression of concentration on time (Fig. 1B), and which were virtually nonexistent.

In view of these findings, as well as the obvious changes in unfrozen samples (room-temperature data, Fig. 1A), the authors are of the opinion that the  $\text{NH}_3\text{-N}$  content of those nearshore and estuarine water samples that are high in ammonia-nitrogen compared to open seawater can be fixed satisfactorily prior to measurement by freezing.

The range of  $\text{NH}_3\text{-N}$  concentration in offshore seawater is about 0.005 to 0.05 ppm (Sverdrup, Johnson, and Fleming 1942). These values are too low to be effectively measured by the analytical method employed here. It is possible that minor changes during cold storage of offshore samples could significantly affect the reliability of later measurements of their  $\text{NH}_3\text{-N}$  content. Further research involving open seawater samples and a more precise method of analysis will have to be conducted before it can be determined if the sample treatment described in this paper can be applied to offshore water.

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